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# Effect of '*Azotobacter*' Bioinoculant on the Growth and Substrate Utilization Potential of *Pleurotus eous* Seed Spawn

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**We investigated the effect of nitrogen fixing *Azotobacter* bioinoculant on the mycelial growth and the rate of substrate utilization by *Pleurotus eous*. The synergistic or antagonistic role of the microorganism during dual culturing with the mushroom or the competitor molds *Trichoderma viride*, and *Trichoderma reesi* was studied. *Azotobacter* was inhibitory to the molds, which are competitive to the mushroom in the seed spawn substrate, but was synergistic towards the mushroom. The growth, substrate utilization potential as total nitrogen content and cellulase enzyme activities of the mushroom in the seed spawn substrate were also enhanced in the presence of the bioinoculant at lower inoculum concentrations, upto 5 *ml* broth culture per spawn bottle.**

**KEYWORDS:** ***Azotobacter*, Bioinoculant, Cellulase, *Pleurotus eous*, *Trichoderma reesi*, *Trichoderma viride***

The major components of lignocellulosic wastes used for mushroom cultivation are cellulose, hemicellulose and lignin. It is estimated that $60\times10^9$ tons of cellulose are generated by photosynthesis each year world wide, but only about 20% are used for conversion into energy and feed. Growth and fruiting of a particular mushroom are dependent on its ability to attack these components as nutrient sources. Nutrition provided by the substrate is the most important factor affecting the yield of mushrooms. Zadrazil (1975) investigating the mycelial growth of *Pleurotus ostreatus* and *Pleurotus florida* reported that higher rates of mycelial growth increased the rate of penetration of the substrate by the fungi, which simplifies overall cultivation. Microbial biomass constitutes 2% of compost weight and act as a concentrated source of nitrogen and minerals for the mushroom mycelium (Wood and Smith, 1988). Post-composting supplementation with proteinaceous materials and phosphate supplementation have been tried to increase the productivity. Thermophilic microbes such as fungi, actinomycetes and bacteria play an important role in preparing the selective substrate for the button mushroom, *Agaricus bisporus* (Adhikary *et al.*, 1992).

Several rhizospheric microbes capable of stimulating plant growth, which are commonly referred to as biofertilizers or microbial inoculants, are being increasingly used in modern intensive cultivation systems. These biofertilizers have been found to stimulate the plant growth through nitrogen fixation, through production of plant growth promoting compounds or by making available nutrients. Some of the nitrogen fixing (diazotrophic) bacteria and pseudomonads also have antagonistic potential against several plant pathogens (Muthukumarasamy *et al.*, 2000). Use of biofertilizers in the mushroom growth media has been investigated for the first time by Ahlawat and Rai (1997).

Since production of quality seed spawn is a crucial step in mushroom cultivation, inoculation of seed spawn substrates with a diazotrophic bacterium is expected to give dual benefits of nitrogen supplementation of the spawn substrate and control of weed fungi. The results reported in this note are the outcome of the study, which tested the effect of '*Azotobacter*' bioinoculant on the growth and substrate utilization potential of *Pleurotus eous* (APK-1) seed spawn and on the common competitor molds found along with it.

## Materials and Methods

**Strains and media.** The biofertilizer '*Azotobacter*' inoculant and the strain of *Pleurotus eous* (APK-1) were obtained from Agriculture College, TNAU, Madurai. In seed spawn cultivation trial, rice hull and rice bran were used. The rice hull was soaked in water for 2 h, drained and autoclaved. The substrate with 60% moisture was mixed with $CaCO_3$ (2% w/w), rice bran (0.6% w/w) and filled in spawn bottles. The mouths of the spawn bottles were plugged with cotton and they were sterilized in an autoclave at 121°C for 2 h. After sterilization, the spawn bottles were inoculated with varying volumes of *Azotobacter* broth culture (2 *ml*, 5 *ml*, 10 *ml* broth culture per bottle) aseptically and mixed thoroughly with a sterile glass rod, followed by inoculation with a 7 mm block from an actively growing mother spawn of *Pleurotus eous*. The uninoculated spawn bottles served as control. For each

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experiment three replicates were maintained.

**Effect of *Azotobacter* sp. on the mycelial growth of *Pleurotus eous*, *Trichoderma viride*, and *T. reesi*.** *Azotobacter* preparation on Jensen's medium (Jensen, 1942) was used for separation of pure and single colonies. Well-separated and distinct colonies were selected for study. Single 7mm bits from actively growing cultures of the mushroom, *Pleurotus eous* or the competitor moulds, *Trichoderma viride* or *Trichoderma reesi*, were used to inoculate individual PDA plates. The bacterial broth culture in log phase of growth was streaked on either side of the fungal agar block simultaneously with the inoculation of the fungus. Petriplates with fungus but without bacteria served as control.

**Effect of *Azotobacter* bioinoculant isolate on the seed spawn of *P. eous*.** The microbial inoculant as log phase broth culture was thoroughly mixed with the spawn substrate using a sterile glass rod in different volumes of 2 *ml*, 5 *ml* and 10 *ml* per bottle and the spawn bottles were inoculated with a 7 mm agar block from an actively growing *P.eous* plate. After plugging the mouth with cotton, the spawn bottles were incubated at 25±2°C. The mycelial proliferation was recorded on the 7th day post-incubation.

**Activities of the enzymes.** Carboxyl methyl cellulase (CMCase) (Padmaja and Balagopal, 1985), filter paper degrading enzyme (FPase), $\beta$-glucosidase (Ray *et al.*, 1993) and total nitrogen content (Jayaraman, 1988) of the substrate were estimated at 7th day and 14th day after incubation.

## Results and Discussion

The isolate from *Azotobacter* bioinoculant was synergistic to the growth of *Pleurotus eous*, but showed antagonism towards *Trichoderma viride* and *Trichoderma reesi* in plate cultures (Fig. 1). *Azotobacter* sp. broth culture at 2 *ml* per bottle concentration increased the linear growth of *P. eous* mushroom. However the increase was neutralized and was brought on par with the control at 5 *ml* per bottle concentration. At 10 *ml* per bottle concentration, there was a decrease in linear growth of the mushroom indicating that the biofertilizer isolate was inhibitory to mycelial growth at higher concentrations (Table 1). These results are in accordance with the earlier work of Ray *et al*. (1993), which reported the role of biofertilizers in suppressing the weed fungi and increasing the mycelial growth of *Agaricus bisporus*.

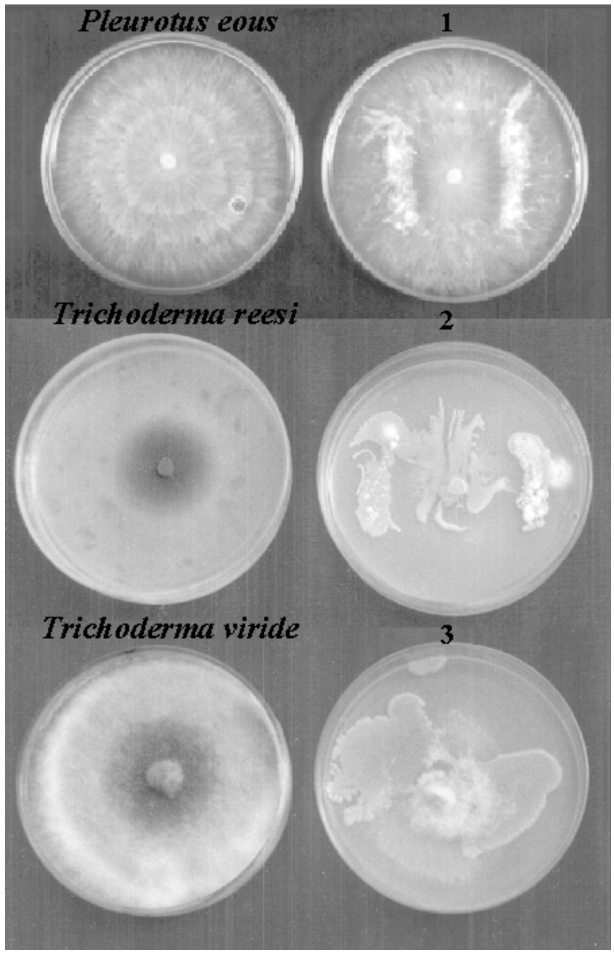


**Fig. 1.** Colony interaction between *Azotobacter* bioinoculant and *Pleurotus eous* (1), *Azotobacter* + *Trichoderma reesi* (2) and *Azotobacter* + *Trichoderma viride* (3).

The activities of the crude cellulolytic enzymes, *viz*., CMCase, filter paper degrading enzyme and $\beta$-glucosi-

**Table 1.** Effect of *Azotobacter* sp. added to seed spawn substrate on mycelial growth of *Pleurotus eous* and substrate total nitrogen content

| Treatment | 7th day Linear growth (cm) | Total nitrogen (mg/g dry wt.) | |
|---|---|---|---|
| | | 7th day | 14th day |
| *P. eous* (Control) | 9.3 | 5.6 | 7.0 |
| *P. eous* + *Azotobacter* (2 *ml* broth per bottle) | 11.0 | 8.4 | 11.2 |
| *P. eous* + *Azotobacter* (5 *ml* broth per bottle) | 9.0 | 7.0 | 9.8 |
| *P. eous* + *Azotobacter* (10 *ml* broth per bottle) | 7.5 | 6.6 | 7.8 |
| LSD (0.05) | 0.875 | 0.535 | 1.268 |

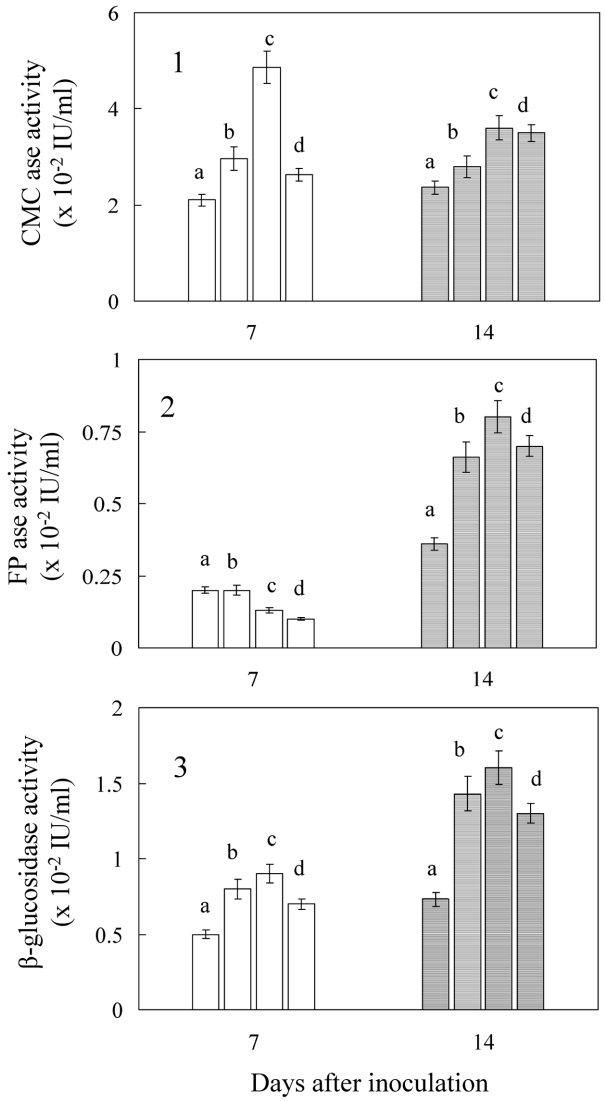


**Fig. 2.** Activity of cellulose degrading enzymes-CMC ase (1), FP ase (2) and $\beta$-glucosidase (3) in the *Pleurotus eous* seed spawn substrate in the presence of *Azotobacter* sp., *P. eous* (control), *P.eous* + *Azotobacter* (2 *ml* broth per bottle), *P. eous* + *Azotobacter* (5 *ml* broth per bottle), *P. eous* + *Azotobacter* (10 *ml* broth per bottle).

dase in the *P. eous* seed spawn substrate were promoted by adding *Azotobacter* isolate (Fig. 2). CMCase activity in the bioinoculant added to substrate was significantly higher than the control on both the 7th and 14th day after inoculation and the quantity of biofertilizer inoculum proportionally increased the cellulolytic activity up to 5 *ml* per bottle concentration. FPase and $\beta$-glucosidase activities were significantly increased over control upon addition of *Azotobacter* isolate on the 14th day but the effect of concentration of the isolate on the enzyme activity was insignificant. The cellulase enzyme activities were lower in the 10 *ml* per bottle treatment than in the 5 *ml* per bottle treatment. Our results are in accordance with those of Singh *et al.* (1994) who observed that *Azotobacter chroococcum* in dual culture with the basidiomycete, *Coprinus fimetarius*, enriched the growth of the fungus by degrading cellulose prior to fungus.

Tan and Wahab (1997) observed that most white rot fungi exhibit low cellulolytic activity in the anamorphic stage and it is known that cultivation conditions influence activity. The presence of a nitrogen-fixing microbe in the spawn substrate might have improved the nutrient requirements of the mushroom, which would have a positive influence on the activity of cellulolytic enzymes. By promoting the cellulolytic activity of the mushroom, the microbial inoculant could direct it towards the production of enzymes targeted at lignin depolymerization, which is coupled to primordia formation. Thus the microbial inoculants at lower concentration might help in earlier pinning and increasing the mushroom yield.

Royse and Bahlar (1988) reported that the nature and amount of nitrogen present in the substrate influenced the degree of cellulose degradation. The results of the present investigation were corroborated by this report. The total nitrogen content of *Pleurotus eous* seed spawn substrate increased during the course of spawn growth as well as in the presence of *Azotobacter* biofertilizer isolate (Table 1). There was a significant increase in total nitrogen content of the substrate up to 5 *ml* per bottle *Azotobacter* isolate treatment indicating its promotive effect on the mushroom growth. At 10 *ml* per bottle *Azotobacter* inoculum concentration, the total nitrogen content of the substrate was lower than that of the other treatments. The linear growth of the mushroom and its cellulolytic activities decreased at the same concentration. This apparent lower nitrogen content of the substrate may be due to the inhibition of substrate mineralization by the fungus at the above-optimal nitrogen content of the substrate at the higher bioinoculant concentration. The change in nitrogen content of the substrate in pure culture mushroom bags was attributed to the degree of substrate mineralization and colonization by the mushroom fungi and the subsequent organic matter loss (Theradimani and Marimuthu, 1992).

The incidence of contamination by molds like *Rhizopus* sp. and *Aspergillus niger* were noticed to be higher in the *P. eous* spawn bottles receiving the microbial inoculants at the higher concentration (10 *ml* per bottle) during visual examination. This is expected, as nitrogen availability in the form of biomass nitrogen increases, the potential for development of infesting weed molds increases. Carroll and Schisler (1976) studying an ideal supplement of mushroom substrate mentioned that excessive heating and stimulation of competitor molds might limit the rate of supplementation and corresponding benefit.

The results of the study indicated that *Azotobacter* bioinoculant was efficient for increasing the growth and sub-

strate utilization potential of the seed spawn of *P. eous* and controlling weed fungal infestation at lower inoculum concentrations. Further work is in progress to study the effect of this microbial inoculant on the yield of *P. eous*.

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